

Appl. No.: 10/657,404
Amtd. Dated: July 7, 2005
Reply to Office Action of February 11, 2005

Amendments to the Specification:

At page 1, please amend the paragraph from line 3 to line 8 as follows:

This application is a continuation-in-part of ~~co~~pending U.S. patent application Ser. No. 09/269,533, now U.S. Patent No. 6,670,452, filed on Jun. 1, 1999, which is a national phase application of PCT/GB97/02610, filed on Sep. 25, 1997, which claims priority from application G9620153.8, filed on Sep. 27, 1996, which are hereby incorporated herein in their entirety by reference.

At pages 21-22, please amend the paragraph from line 25 of page 21 to line 20 of page 22 as follows:

Egg yolk phosphatidylcholine (PC) was purchased from Lipid Products, Surrey, United Kingdom and used without further purification (>99% pure). Tris (hydroxymethyl)-methylamine (Tris TRIS®), hydrochloric acid (HCl), dimethylsulfoxide (DMSO) and t-butanol (all Analar grade) were obtained from Merck Ltd, Glasgow, United Kingdom. Triolein (99% pure, TO), cholesteryl oleate (98% pure, CO), phosphate buffered saline (PBS) tablets and the reagents used for cholesterol testing were purchased from the Sigma Chemical Company, Dorset, United Kingdom. The polycarbonate filters used in the extrusion process were obtained from Costar Corporation, Buckinghamshire, United Kingdom. Sterile, disposable 0.2 µm polysulfone filters used for filter sterilisation were purchased from Whatman Ltd, Maidstone, United Kingdom. Sterile, distilled water complying with the European Pharmacopoeia's water for injection monograph was purchased from Steripak Ltd, Runcorn, United Kingdom. All synthetic polypeptides were synthesised at Thistle Peptide Services (formerly BioMac) at the Department of Biochemistry, University of Glasgow. Peptide structures are listed in FIG. 7, material was supplied at greater than 90% purity and was used as received. All tissue culture materials (media, serum, culture flasks, pipettes and multi-well plates) were obtained from Life Technologies Ltd., Paisley, United Kingdom. U937 cells were obtained from the European Collection of Animal Cell Cultures, Salisbury, United Kingdom. DFCS was prepared from FCS by the method of Rothblat et al.⁶ Monoclonal anti-LDL receptor antibody (clone C7, code number RPN.537) was purchased from Amersham International and reconstituted with sterile water just prior to use.

At pages 22-23, please amend the paragraph from line 26 of page 22 to line 21 of page 23 as follows:

A 3:2:1 molar mixture of Phosphatidylcholine: Triolein: Cholesteryl Oleate (PC:TO:CO) was dissolved in chloroform/methanol 2:1 (V/V) and the solvent removed under a stream of nitrogen. The

lipids were then re-dissolved and lyophilised from t-butanol for 24 hours (EF4 Modulyo Freeze Dryer, Edwards High Vacuum, Crawley, United Kingdom), then re-suspended in 0.01M Tris-HCl TRIS®-HCl buffer (pH 8.0) to give a final concentration of 7 to 8% w/v for extrusion. The lipid dispersions were sonicated under a stream of N₂ for two hours using a 250W sonicator, centrifuged at 10,000 rpm for 60 minutes (MSE Superspeed 75 Ultracentrifuge, MSE Ltd, London, United Kingdom) and then transferred to the Extruder vessel (Lipex Biomembranes Inc, Vancouver, Canada) which was maintained at 50-55° C throughout. The lipid mixture was successively extruded through polycarbonate filters of pore size 0.1 and 0.05 µm using two stacked filters and at least four extrusions under 60 psig pressure provided by a nitrogen source⁸. Samples of lipid microemulsion were diluted with Tris-HCl TRIS®-HCl buffer to give a cholesterol concentration of approximately 1 mmol/l and heated to 55° C in a stirring water bath. Aliquots of peptide dissolved in DMSO were added under the surface of the stirring microemulsion, control experiments were performed with DMSO alone. The volume of DMSO added in each case was kept below 20 µl/ml of microemulsion mixture. The peptide-microemulsion complex was incubated at 55° C for 15 minutes then dialysed overnight against 5 litre PBS. The resulting non-naturally occurring LDL (nLDL) were filter sterilised (0.2 µm) and stored at 4° C under N₂ before use.

At page 25, please amend the paragraph from line 21 to line 28 as follows:

To 3 ml of phospholipid reagent (phospholipase D, choline oxidase, peroxidase, 4-aminoantipyrine, Tris TRIS® buffer, calcium chloride, phenol) was added 0.020 ml of sample or standard (choline chloride and phenol) concentration 300 mg/100 ml. The mixture was incubated at 37°C for 10 minutes 10 minutes. The absorbance of each sample was measured spectrophotometrically at 505 nm. Phospholipid content was calculated by reference to a phospholipid standard.

At pages 27-28, please amend the paragraph from line 22 of page 27 to line 2 of page 28 as follows:

Particle size analysis was carried out using photon correlation spectroscopy (Zetasizer ZETASIZER® 4, Malvern Instruments, Malvern, UK). Before analysis samples were diluted with Tris-HCl TRIS®-HCl buffer (0.01M) and filtered (0.2 µm). Sizing measurements were carried out at a fixed angle of 90°. The correlator was operated in parallel mode and the cumulants method of analysis was used to calculate the mean sample size weighted according to the intensity of scattered light (z-average diameter). Since this diameter is weighted strongly in favour of large particles, Rayleigh theory was used to convert intensity distributions into number distribution.

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At page 28, please amend the paragraph from line 5 to line 9 as follows:

Samples were diluted 1 in 5 with 0.01M Tris TRIS[®] buffer (pH 8.0) and Zeta potential measured at 25° C using a Zetasizer ZETASIZER[®] 4 (Malvern Instruments). The applied voltage was 150V in each case and duty cycling was used to limit the cell current to 20 mA.